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TRANSPORT OF SUGARS IN THE TAPEWORM *CALLIOBOTHRIUM VERTICILLATUM* [1]

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Since tapeworms have no digestive tract and no cavities in the body other than the so-called osmoregulatory canals and genital ducts, it has been assumed that nutrients enter the body through the outer surface. Further, several tapeworm species are known to require an external source of carbohydrate for growth and reproduction; at least 14 species can metabolize no sugars other than glucose and galactose (Read and Simmons, 1963). *Calliobothrium verticillatum*, a tetraphyllidean cestode parasitizing the smooth dogfish, has been reported to absorb and metabolize glucose and galactose but not to utilize fructose, mannose, sucrose, lactose, trehalose, or maltose from the suspending medium (Read, 1957; Laurie, 1961). It seems highly probable that glucose, and perhaps galactose, is a required energy source for *Calliobothrium*. Although no data on the concentrations of free monosaccharides in the environment of the worm are available, it seemed desirable to study sugar absorption by the worm *in vitro*, with the view that sugar metabolism may limit growth and reproduction of *Calliobothrium* in its host.

Materials and Methods

The hosts, *Mustelus canis*, were collected by a commercial fisherman and distributed by the Supply Department of the Marine Biological Laboratory. The dogfish were maintained in large tanks with running sea water (18–22°) until the time of an experiment. The holding period varied from one to three days. The hosts were killed by a blow on the chondrocranium and the spiral intestine quickly removed to a container immersed in ice. The intestinal valves were split longitudinally and the adult cestodes removed to a balanced salt solution also maintained at 0° (250 m*M* NaCl, 4.4 m*M* KCl, 5.1 m*M* $CaCl_2$, 2.9 m*M* $MgCl_2$, and 300 m*M* urea, buffered with 10 m*M* tris-maleate at pH 7.2, as described by Read, Simmons, Campbell and Rothman 1960). This solution is referred to herein as saline. The parasites were freed of adhering intestinal contents and mucus and sorted into groups of about eight to ten worms. Each sample was placed in 4 ml of saline in a 30 ml beaker. Beakers containing the parasites were preincubated in a shaking water bath at 20° for 30 minutes. Unless otherwise stated, all incubations were carried out at 20° C. At the end of an incubation, worms were rapidly removed from the incubation medium, quickly rinsed three times in saline, blotted on hard filter paper, and placed in 2 ml of 70% ethanol. Extraction of soluble materials was carried out at room temperature for at least

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18 hours with frequent agitation. Incubation times and other manipulations will be discussed in the context of the experiments.

Wet weights of parasites were determined on a torsion balance. Dry weights and ethanol-extracted dry weights were obtained after drying the material to constant weight at 103° C in small tared aluminum cups. Estimation of parasite body water was made gravimetrically, *i.e.,* wet weight minus dry weight.

Isotopic methods

Radiochemicals were obtained from New England Nuclear Corporation and Cambridge Nuclear Corporation. Radioactivity was determined on aliquots of ethanol extracts or incubation media using a low background gas flow counter (C^{14}) or a gamma ray spectrometer with a NaI crystal (K^{42} and Na^{24}). Specific activities (c/m μmole^{-1} or c/m meq^{-1}) were determined by counting aliquots of various media after appropriate dilution with 70% ethanol.

Chemical methods

All unlabeled carbohydrates were purchased from commercial sources (Mann Research Laboratories, Pfanstiehl Chemical Co. and Sigma Chemical Co.).

Polysaccharide and total ethanol-soluble carbohydrate were determined by the phenol-sulfuric acid method of Dubois, Gilles, Hamilton, Rebers and Smith (1956). Polysaccharide was insolated by digestion at 100° C in 30% KOH and precipitation with 1.2 volumes of 95% ethanol. For determination of the specific activity of the polysaccharide (μmoles ^{14}C-glucose incorporated/μmole glucose in polysaccharide), the precipitated material was washed five times with 70% ethanol containing 0.1% LiCl, by alternate dispersion and centrifugation. This was found to be necessary for the complete removal of ethanol-soluble radioactivity. The washed pellet was then dissolved in water and aliquots removed for the determination of radioactivity and total carbohydrate, using glucose or trehalose standards.

Glucose was determined on aliquots of extracts or media by the glucose oxidase method ("Special Glucostat," Worthington Biochemical Corp.). The purified enzyme preparation was employed to minimize errors due to other carbohydrates in the experimental materials.

Sodium and potassium were determined by flame photometry, using a Coleman Junior spectrophotometer with a flame attachment. Standard solutions contained equivalent amounts of each ion in the chloride form and ethanol at the same concentration as samples. All inorganic reagents were obtained from Fisher Scientific Co. or J. T. Baker Chemical Co.

Results

Effects of the gas phase

Since carbon dioxide is known to affect carbohydrate metabolism in a number of animal parasites (von Brand, 1966; Prichard and Schofield, 1968; McDaniel, Read and MacInnis, in press), it seemed advisable to determine whether the gas phase affected the absorption of glucose in *Calliobothrium.* Data from such experiments are shown in Figures 1 and 2. When compared with the results

obtained in air, nitrogen-carbon dioxide had no significant effect on the absorption or accumulation of glucose, but had a dramatic effect on the incorporation of ^{14}C-glucose into glycogen. This effect is clearly due to the presence of carbon dioxide in the atmosphere since McDaniel *et al.* (in press) showed that under

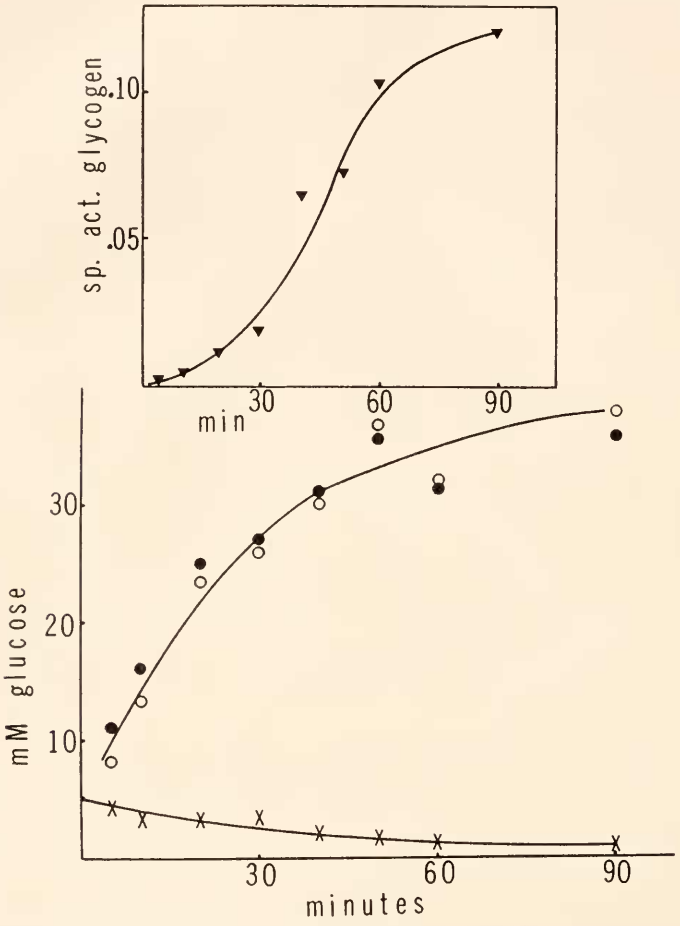

FIGURE 1. The absorption of ^{14}C-glucose by *Calliobothrium* under atmosphere of 95% N_2–5% CO_2. Bicarbonate was added to maintain pH at 7.2. Glucose in worms was determined by glucose oxidase (●) or by radioactivity (○). Glucose in the medium was determined by glucose oxidase (×). Inset's: Incorporation of ^{14}C-glucose into glycogen in same worm samples expressed as specific activity (micromoles ^{14}C-glucose per micromole of glycogen glucose). Each point in all curves is the average of worm samples. Compare with Figure 2.

nitrogen or carbon dioxide-free air, incorporation of ^{14}C-glucose into the glycogen of *Calliobothrium* was markedly less than that observed when the gas phase contained carbon dioxide. Since absorption was not so affected, subsequent experiments were carried out in air. It may be pointed out that, under the conditions of the experiments shown in Figures 1 and 2, the kinetics of absorption were

first order. Further, at concentrations which probably resemble those of the worm's environment, the worm clearly accumulates glucose against a concentration difference (Table 1). Later experiments were designed to determine whether or not this was a saturable system.

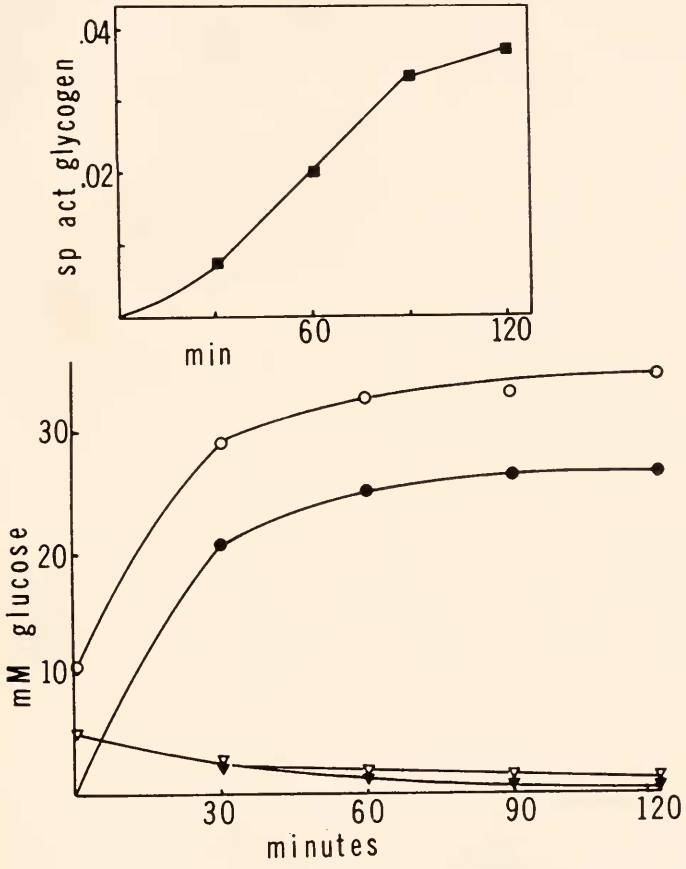

FIGURE 2. The absorption of ^{14}C-glucose by *Calliobothrium* under atmosphere of air Glucose in worms determined by glucose oxidase (○) or by radioactivity (●). Similarly, glucose in the medium was determined by glucose oxidase (▲) or by radioactivity (△). Inset is: Incorporation of ^{14}C-glucose into glycogen in same worm samples expressed as specific activity (micromoles ^{14}C-glucose per micromole of glycogen glucose). Each point in all curves is the average of two worm samples. Compare these data with Figure 1.

Effect of temperature

When worms were incubated with glucose at each of several temperatures for 60 minutes, sugar accumulation rose sharply with temperature to a maximum at about 20° C. Above 30° C, absorption sharply declined (Fig. 3). The data do not allow a very precise estimate of the optimum temperature for glucose accumulation, but the extent to which glucose is incorporated into glycogen appears to have a higher temperature optimum than that for glucose accumulation (Fig. 3).

TABLE I

Effect of time on the accumulation of glucose by Calliobothrium. *Incubation mixture contained 5 ml of 0.5 mM glucose in KRT. Results based on average of two determinations*

Incubation time (minutes)	Glucose conc. inside (m*M*)	Glucose conc. outside (m*M*)	$\frac{\text{Conc.}_i}{\text{Conc.}_o}$
0	4.9	0.5	9.8
1	5.3	0.46	11.5
2	6.8	0.43	15.8
4	7.7	0.38	20.3
6	8.5	0.36	23.5
8	9.0	0.29	31.0
10	10.4	0.28	37.0
20	12.1	0.12	100.8
30	12.6	0.04	315.0

Effect of pH

The absorption of glucose was affected by hydrogen ion concentration; the highest rates were observed at about pH 8 to 9 (Fig. 4). There was a dramatic

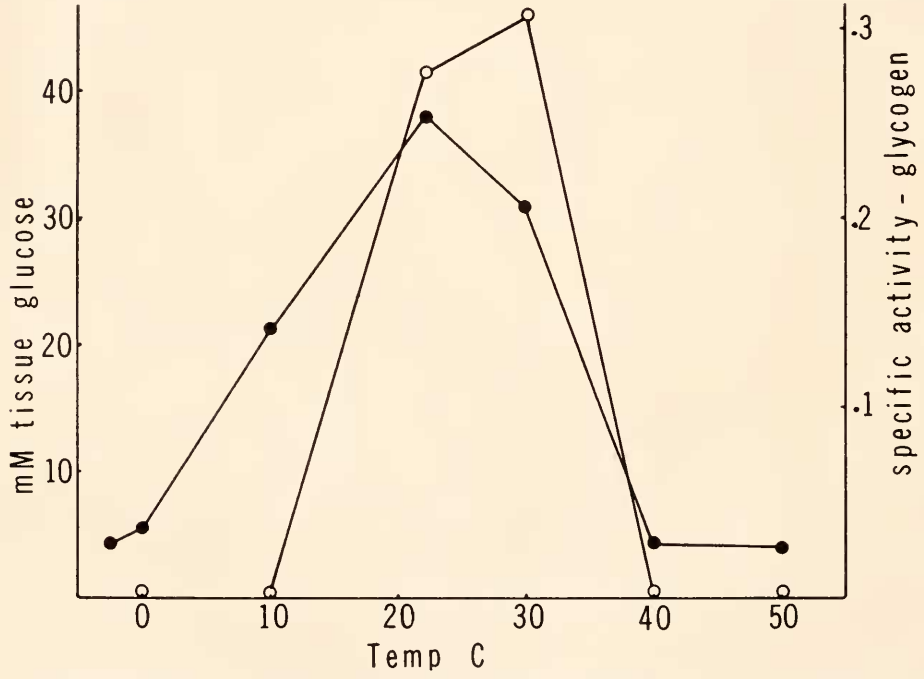

FIGURE 3. Effect of temperature on the 60 minute accumulation of tissue glucose (●) and on incorporation of ^{14}C-glucose into glycogen (○). Tissue glucose was determined by glucose oxidase. Specific activity is μmoles ^{14}C-glucose per μmole of glycogen glucose. The medium contained 5 m*M* glucose at the beginning of the experiment. Each point is the average of two samples.

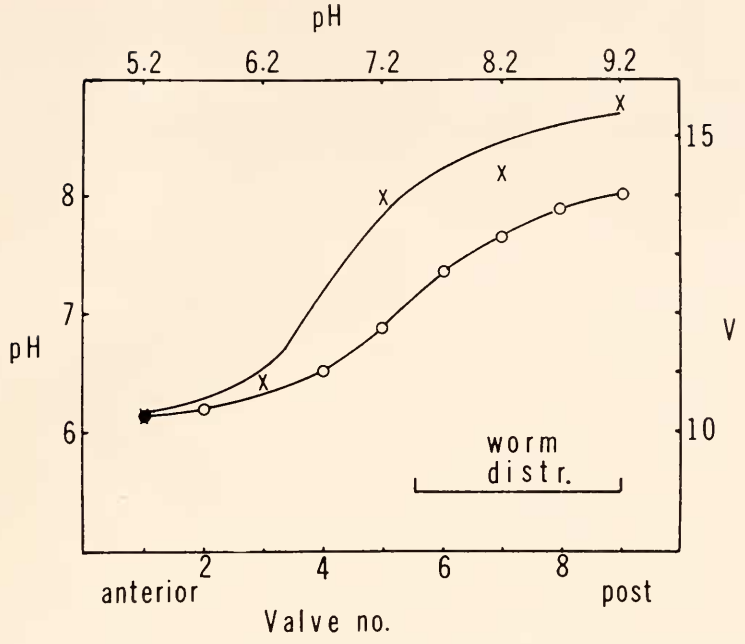

FIGURE 4. Effect of pH on rate of ^{14}C-glucose absorption in 2 min incubations (×) and the pH of intestinal contents in different segments of the dogfish spiral intestine (○). Worm samples were preincubated for 60 minutes in buffered saline at the appropriate pH and then incubated with 0.01 m*M* ^{14}C-glucose at the same pH as the preincubation medium. The V = μmoles per gram dry wt per hr. Normal worm distribution in the spiral intestine is indicated.

TABLE II

The effect of various compounds on the transport of glucose by Calliobothrium. *Inhibitor concentration, 5 m*M; *substrate concentration, 0.05 m*M: *Incubation time 2 min*

Inhibitor	% Inhib.	No inhibitory effect	
Glucose	75.6	n-acetyl-glucosamine	alanine
Arbutin	73.2	D-glucosamine	arginine
Maltose	59.9	lactose	aspartic acid
α-Methylglucoside	58.6	fructose	glycine
Galactose	44.2	mannose	leucine
Cellobiose	25.2	3-0-methylglucoside	methionine
Salicin	23.2	rhamnose	proline
Ouabain*	24.5	sorbose	taurine
Phlorizin**	96	sucrose	trimethylamine
			valine

* Present at 2 m*M*.
** Present at 0.01 m*M*.

drop in the entry kinetics between 7.2 and 6.2. Determination of the pH in various segments of the spiral intestine of *Mustelus* revealed that there is a pH gradient. In the region of the first spiral, the pH is about 6 and rises to a pH of about 8 in the ninth spiral. These data are shown in Figure 4 and the observed distribution of *Calliobothrium* strobilae in the spiral intestine is shown

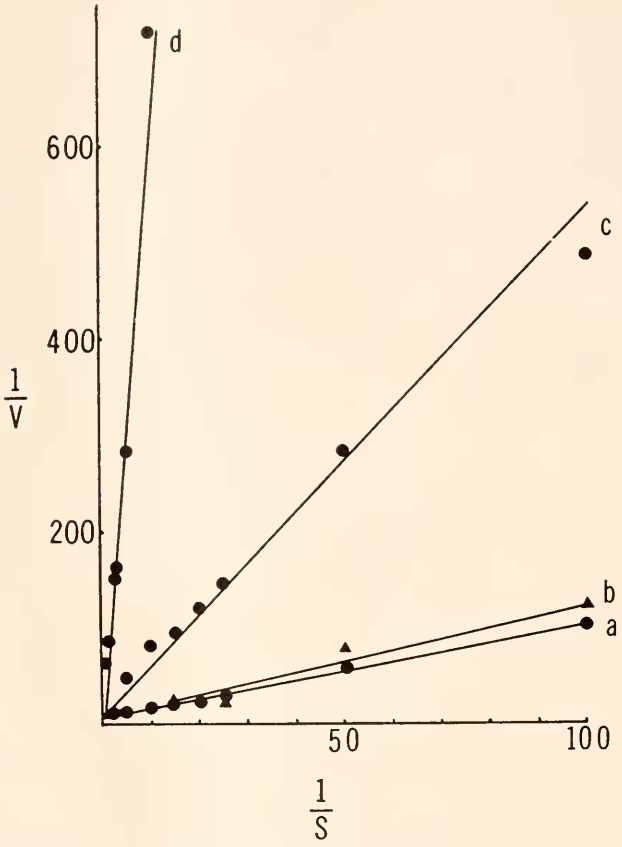

FIGURE 5. Lineweaver-Burke plots of ^{14}C-glucose absorption in 2-min incubations without inhibitor (a) or with 0.01 m*M* phloretin (b) or 0.01 m*M* phlorizin (d). Samples of curve c were incubated for 60 minutes in 0.01 m*M* phlorizin, rinsed, and incubated for 2 min with ^{14}C-glucose. Each point is average of two samples. Abbreviations are: V = μmoles per gram per hour; S = m*M* ^{14}C-glucose.

for comparative purposes. The worm appears to inhabit those segments of the spiral intestine having a pH range which is highly favorable for the absorption of glucose by the parasite. It would be of interest to examine the effect of pH on the absorption of glucose by free segments of *Calliobothrium,* since these are normally found in the first two spirals where the pH is relatively low. Attempts to make such determinations have proven to be technically difficult.

Effects of inhibitors

In 2 min incubations, 5 m*M* 2,4-dinitrophenol had no effect on the rate of glucose transport. However, when the worms were preincubated for 10 min in 5 m*M* dinitrophenol, glucose absorption was inhibited 30% in a subsequent 2 min incubation without dinitrophenol. Sodium iodoacetate at 0.5 m*M* produced a 30% inhibition of glucose transport in 2 min incubations.

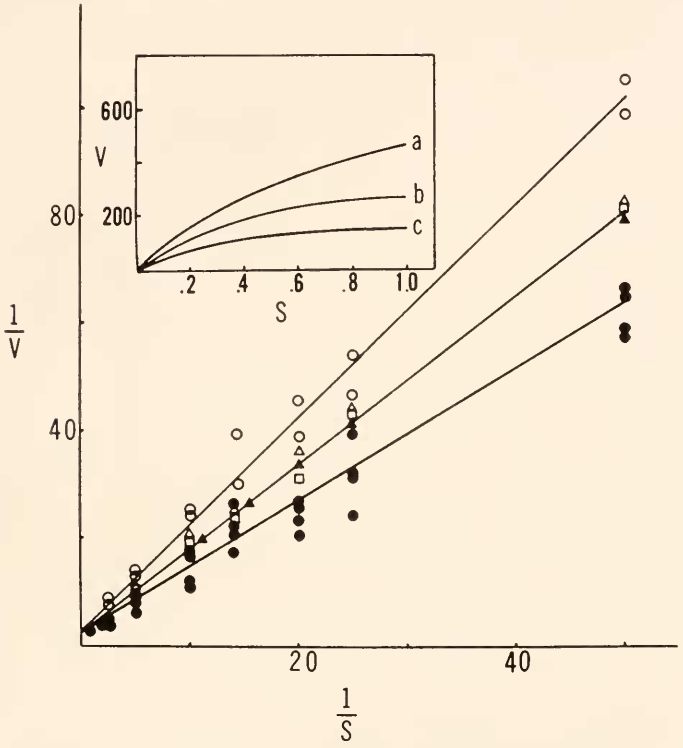

FIGURE 6. Lineweaver-Burke plots of ^{14}C-glucose absorption in 2-min incubations in the presence of galactose (△), maltose (□), α-methylglucoside (▲), ouabain (○), or without inhibitor (●). Inhibitors were present at 2 m*M*. Points for curves with galactose, maltose, and α-methylglucoside as inhibitors are averages of two determinations while those of the ouabain-inhibited and uninhibited glucose absorption curves are individual determinations. Inset is: Curves for absorption of glucose alone (a), glucose in the presence of 2 m*M* maltose (b), and glucose in the presence of 2 m*M* ouabain (c). Data points from the lower curves were used to construct inset curves. Abbreviations are: S = m*M* glucose; V = μmoles absorbed per gram dry weight per hour.

Twenty-eight additional compounds were examined as inhibitors of glucose transport (Table II). Of those tested, eight produced significant inhibitions. When these same compounds were tested as stimulators of the efflux of previously accumulated glucose, only those which inhibited glucose absorption caused an enhanced leakage of glucose. Further, sodium α-ketoglutarate, β-glycerophosphate, or dithiazanine had no effect on efflux of previously accumulated glucose.

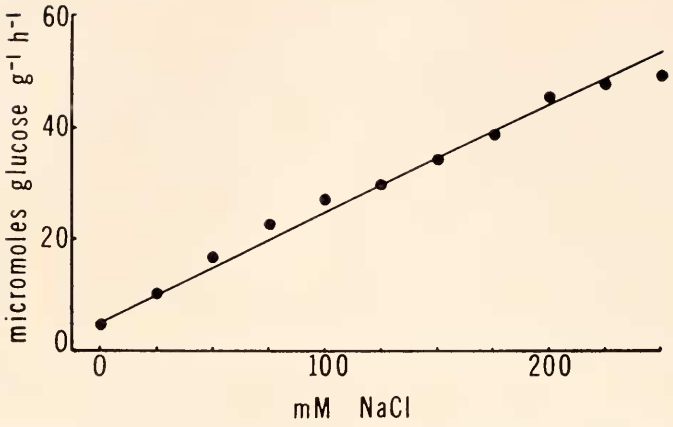

FIGURE 7. Effect of Na^+ on the absorption of ^{14}C-glucose in 2-min incubation. Deleted NaCl was replaced by KCl. Glucose was present at 0.2 m*M* in all incubates and each point is average of two samples.

The latter compound is an anthelminthic drug which blocks sugar transport in the isolated gut of *Ascaris* (Fisher, unpublished) but has no effect on glucose uptake or efflux in *Calliobothrium*.

Phlorizin, which is known to be a strong inhibitor of glucose transport in other tapeworms (Phifer, 1960; Laurie, 1961), as well as various other cells and tissues, was the most powerful inhibitor of glucose transport in *Calliobothrium*. The inhibition appeared to be competitive in character in 2 min incubations (Fig. 5). However, when worms were incubated for 60 min in the presence of 0.01 m*M* phlorizin and rinsed in phlorizin-free saline, the inhibition of glucose uptake was only partially reversed (Fig. 5c). The effect of phlorizin is apparently dependent on the intact glycoside since the aglycone, phloretin, produced a negligible inhibition (Fig. 5b).

Ouabain, a cardiac glycoside, produced a significant inhibition of glucose transport, but the glycone moiety of ouabain, rhamnose, was not inhibitory (Table II). Two other glycosides, arbutin and salicin, had significant activity as inhibitors of glucose transport (Table II).

The inhibitions produced by galactose, α-methylglucoside, maltose, and ouabain were examined in some detail. Lineweaver-Burke plots of the data indicated that the inhibitions are competitive in character (Fig. 6).

TABLE III

Effect of glucose entry on the ionic composition and wet weight of Calliobothrium

Incubation time (minutes)	K^+ meq. l^{-1}	Na^+ meq. l^{-1}	Wet weight increase (mgm%)
0	87	172	—
30	79	203	7.3
60	73	221	13.5
90	71	229	19.5
120	70	234	32.2

Na and K Effects

Since sodium has been implicated in the transport of sugars and amino acids in other animal cells (Stein, 1967), it was deemed desirable to determine whether sodium is involved in glucose transport in *Calliobothrium.* When K^+ was substituted for Na^+ in the saline, the rate of glucose transport was a linear function of Na concentration (Fig. 7). Further, it was found that tissue sodium increased when glucose was being absorbed, as might be expected in the co-transport of sodium and glucose. The increase in sodium was accompanied by less than compensatory decrease in tissue potassium and by an increase in tissue water (Table III). Control worms incubated in saline without glucose showed no significant changes in tissue sodium, potassium, or wet weight.

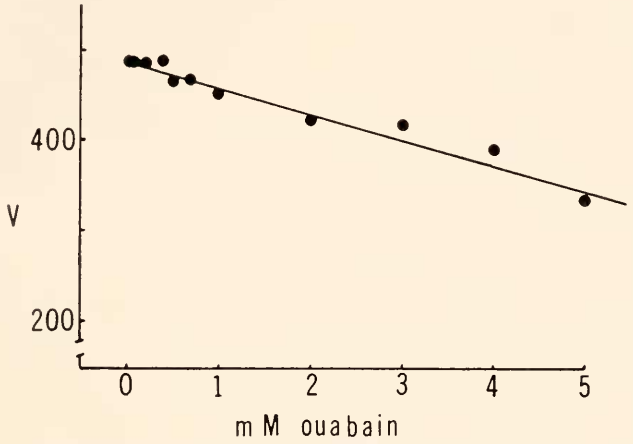

FIGURE 8. The effect of various concentrations of ouabain on the absorption of 5 m*M* ^{14}C-glucose in 2-min incubations of *Calliobothrium.* The V = μmoles absorbed per gram dry weight per hour.

As previously noted, the glycoside ouabain inhibits glucose transport in *Calliobothrium.* In 2 min incubations, the inhibition increases linearly between 0 and 5 m*M* ouabain (Fig. 8). This inhibitor is known to act on sodium transport in a number of animal cells, blocking the sodium extrusion mechanism. In *Calliobothrium,* ouabain causes a marked net influx of sodium (Fig. 9). Exposure to ouabain causes the tissue sodium to rise from about 180 to 300 meq/l in 30 minutes. This is accompanied by an enhanced net efflux of potassium which is not equivalent to sodium influx. Glucose partially alleviates the effect of ouabain on the fluxes of both sodium and potassium. It may also be noted in Figure 8 that ouabain inhibits the net accumulation of glucose by *Calliobothrium,* reducing it by about 63%.

If ouabain acts on the system involved in maintaining sodium at internal concentrations which are lower than that of the ambient medium, it might be expected to affect efflux of sodium from the worm. To test this hypothesis, worms were equilibrated for 60 min in saline containing Na^{24}, following which the worm samples were transferred repetitively at 5 min intervals to vessels

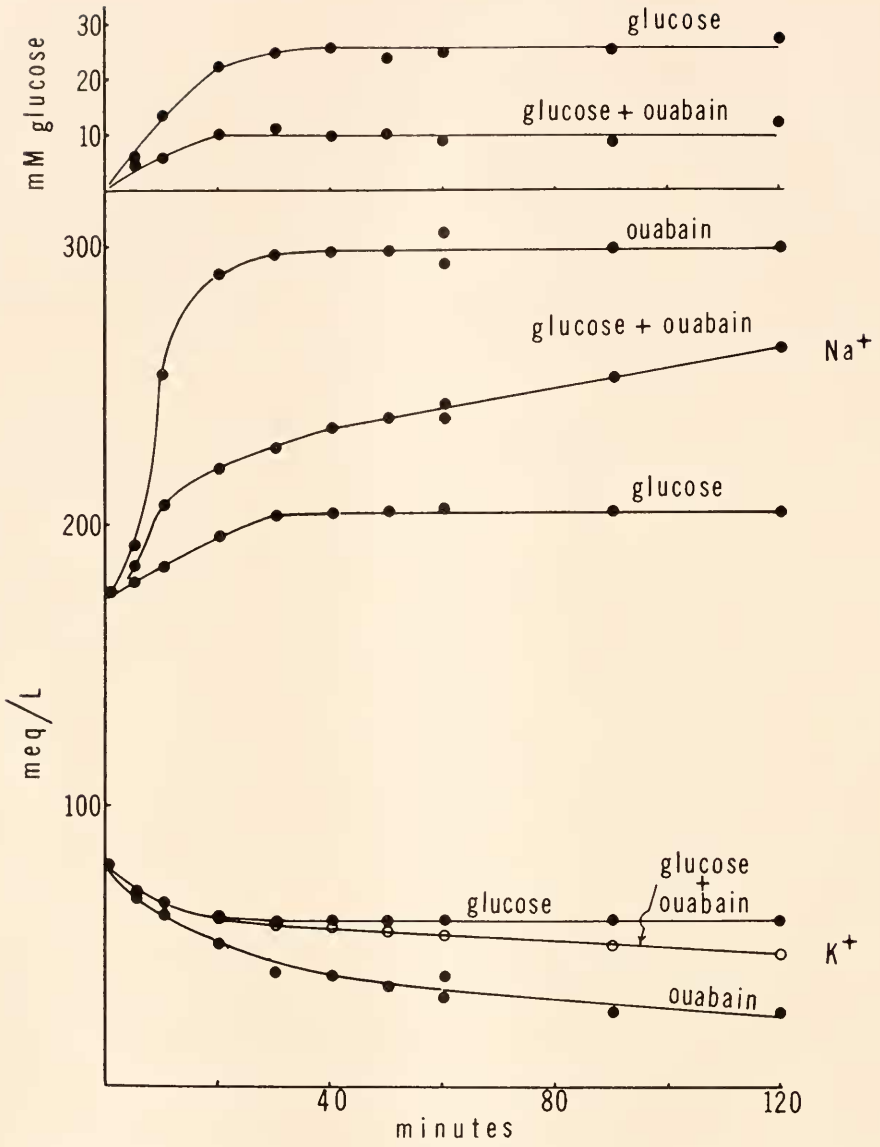

FIGURE 9. Effect of ouabain on Na^+ and K^+ net fluxes and on the accumulation of glucose. Glucose and ouabain at concentration of 5 m*M*. All values are expressed in terms of tissue water and each point is average of two samples. Controls incubated without glucose and/or ouabain showed no net change in Na^+ or K^+ levels during the same time period.

containing saline with or without 5 m*M* ouabain. The media were then assayed for Na^{24}. Results of such an experiment are shown in Figure 10. The data indicate that sodium effluxes from at least two compartments. Ouabain appears to exert an effect on one compartment and not on the other. Efflux from the

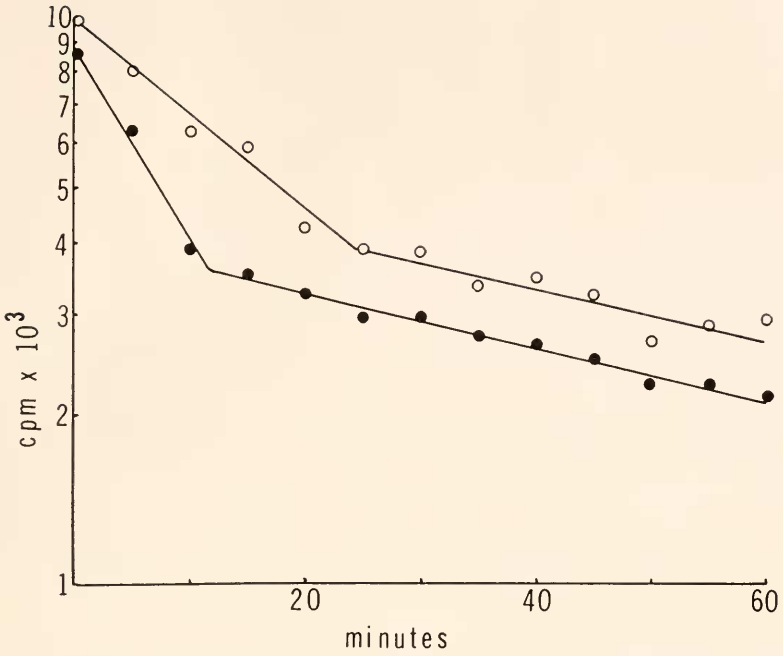

FIGURE 10. Effect of ouabain on efflux of Na^+. Worms were equilibrated for 60 min in saline containing Na^{24}. Efflux was then measured for 60 min in the presence (○) or absence (●) of 5 m*M* ouabain. See text for experimental details.

"fast compartment" is clearly inhibited by ouabain, whereas efflux rates from the other compartment appear to be the same for ouabain-treated and control samples.

Accumulation of other hexoses

Since glucose was accumulated against a concentration difference, it was of interest to determine whether some other hexoses are accumulated by *Calliobothrium* and to examine the relative rates at which these hexoses might be incorporated into parasite polysaccharide. Such data are presented in Table VI.

Of those examined, galactose and glucose are accumulated against a concentration difference. Although a specific galactose oxidase was not employed to examine the levels of galactose in the worm, the amount of non-glucose, ethanol-soluble carbohydrate (Table IV, column d) is about four times that found in worms incubated with other sugars. It is concluded that a large proportion of this non-glucose carbohydrate is indeed galactose. Although galactose is transported, very little of this sugar is incorporated into polysaccharide (Table IV, column g). Mannose and fructose are not transported and, as anticipated, negligible amounts of label from these sugars are incorporated into polysaccharide. Additional experiments on the absorption of 3-0-methylglucose showed that the rate is a linear function of sugar concentration and that it is neither accumulated against a concentration difference nor metabolized by the worm.

The data indicate that 3–0-methylglucose enters the worm by diffusion and substantiates the finding that this sugar does not react with the glucose transport system.

Discussion

The outer surface of the syncytial tegument of *Calliobothrium* is elaborately differentiated for absorptive function, as is the case in all cestodes whose tegumentary ultrastructure has been studied (Lumsden, 1966). As we have shown, glucose is absorbed at high rates by the worm and, at concentrations which probably fall in the range normally encountered by the worm in its environment, *Calliobothrium* accumulates glucose against a concentration difference of up to 315 fold in a 30 minute period. The capacity of the worm to

Table IV

Accumulation of radioactive hexoses by Calliobothrium *and incorporation into polysaccharide; incubation time: 60 min, incubation medium: 4.0 ml of 5.0 m*M *substrate; average of 2 samples/hexose; gas phase: 95% N_2–5% CO_2*

Subst.	C^{14} ("m*M*") (a)	Gluc. (m*M*) (b)	EtOH$^{sol.}$ CHO ("m*M*") (c)	Non Gluc. CHO ("m*M*") (d)	Medium Gluc. (m*M*) (e)	Medium Total CHO ("m*M*") (f)	Sp. act. Polysac. (g)
Gluc	46.78	31.23	38.60	7.37	3.84	3.94	0.1248
Gala	66.37	11.27	59.89	48.62	—	2.00	0.0011
Mann	2.16	12.94	24.67	11.73	—	5.01	0.0004
Lev	1.02	11.55	23.61	11.61	—	5.01	0.0001
	—	8.87	23.51	14.64	—	—	—

(a) Based on specific activity of medium.
(b) Determined by glucose oxidase.
(c) Determined by phenol: H_2SO_4.
(d) Determined by difference in, c − b.
(e) Determined by glucose oxidase.
(f) Determined by phenol: H_2SO_4.
(g) μMoles C^{14} substrate incorporated/μmole hexose in polysaccharide.

accumulate glucose is not affected by the presence of carbon dioxide or bicarbonate in the medium, although carbon dioxide clearly affects the rate at which glucose is incorporated into glycogen. This is similar to results obtained with the cyclophyllidean tapeworm *Hymenolepis diminuta* (Fisher and Read, unpublished data).

The temperature optimum for glucose absorption is perhaps slightly higher than that normally prevailing in the marine environment of the host. However, the body temperature of an active dogfish may be slightly higher than that of the surrounding sea water and it seems probable that the worm in its host would absorb glucose at something approaching the maximum rate at a given concentration.

There is a high correlation between the pH in that part of the spiral intestine inhabited by *Calliobothrium* and the pH values at which maximum rates of glucose absorption were observed. In the anterior part of the spiral intestine,

not inhibited by the strobila of *Calliobothrium,* the prevailing pH would reduce glucose absorption by 30 to 35%. This might be of significance in allowing normal function of the worm. On the other hand, the free segments of *Calliobothrium* which are in varying stages of shelled egg development are typically found in the two anterior valves of the spiral intestine. The energy requirements of the free segments may be considerably less than the requirements of the strobila, since active tissue growth must be occurring at lower rates.

The inhibition produced by dinitrophenol clearly does not occur at the surface since it produced no inhibition in 2 min incubations. It may produce its effect over longer periods of exposure by general interference with water and salt movements. Iodoacetate, on the other hand, is a very non-specific poison and seems to produce an effect on the initial rates of glucose absorption probably combining with the carrier itself.

Of other potential inhibitors tested, only a limited number of hexoses and glycosides were inhibitory. None of the amino acids and several sugars were not inhibitory. Of the inhibitory sugars, five were found to produce inhibition in a competitive manner. The others were not examined with respect to this point. However, the observed competitive inhibitions of glucose uptake by other sugars; including the lack of inhibition by 3–0-methylglucoside and the inhibition by cellobiose and maltose suggest that the specificity of the site for glucose attachment to the transport mechanism in *Celliobothrium* differs from that in hamster mucosal cells (Crane, 1960) and in the tapeworm *Hymenolepis diminuta* (Read, 1961). Study of possible inhibitory effects of larger series of sugars, particularly hexoses, is required for definition of the points of glucose attachment in the transport mechanism. The lack of interaction by 3–0-methylglucose suggests that the third carbon on the hexose molecule is involved in glucose interaction. The finding that 3–0-methylglucose appears to enter the worm by diffusion, rather than by a mediated process, independently supports the concept that this sugar cannot react with the glucose transport system.

The finding that maltose and cellobiose inhibit glucose uptake must be interpreted with caution. It would be unwise to assume that the inhibitory effects are produced by the intact disaccharides. It is well known that disaccharidases are membrane-bound and act at the mucosal cell surface in the vertebrate intestine (Crane, 1968). The tapeworm surface has been described as a digestive-absorptive surface and, in the case of *Hymenolepis diminuta,* has been shown to release the products of hexose phosphate hydrolysis in the external medium (Arme and Read, 1970; Dike and Read, in press). Lumsden, Gonzalez, Mills and Viles (1968) also described an ATPase acting at the outer boundary of the tegument in *H. diminuta.* Further, the rabbit tapeworm *Cittotaenia* has a membrane-bound disaccharidase which hydrolyzes sucrose, liberating fructose into the external medium (Read and Rothman, 1958). Hence, there is a possibility that the apparent inhibition of glucose transport in *Calliobothrium* by maltose and cellobiose might be due to effects of glucose liberated by the action of intrinsic membrane-bound disaccharidases situated in close proximity to the glucose transport system in the outer face of the *Calliobothrium* tegument. The results of our experiments in which maltose in the external medium appeared to produce enhanced efflux of previously accumulated glucose might indicate the action of a

surface maltase. In studies to be published elsewhere, Fisher has shown that *Calliobothrium* does not secrete a maltase into the external medium. Clearly, the inhibition of glucose transport by maltose and cellobiose requires further study.

Of the glycosides tested, phlorizin was the strongest inhibitor of glucose transport. Laurie (1961) reported that phloretin, the aglycone moiety of phlorizin, was a strong inhibitor of glucose absorption. We have not verified this. However, Laurie's experiments were of 1 to 4 hour duration, while those of the present work were 2 min incubations. Phloretin may affect glucose accumulation rather than the initial rate of glucose transport in *Calliobothrium.*

The changes in tissue sodium occurring with glucose absorption strongly suggest that glucose and sodium enter this worm by co-transport. Since sodium seems to activate glucose transport as an arithmetically linear function of sodium concentration, it may be concluded that reaction of only one sodium ion is required to activate the system for the transport of a glucose molecule. The data also suggest that a change in transmembrane electric potential should accompany glucose transport in *Calliobothrium,* but this has not been measured. The effects of ouabain on sugar transport and sodium fluxes furnish additional evidence that sodium is involved in glucose transport and glucose accumulation. The results seem to be completely consistent with Crane's view that glucose accumulation in vertebrate cells is attributable to the maintenance of low intracellular sodium, with a resulting lower affinity of the membrane carrier for sugar on the intracellular aspect of the cell boundary (Crane, 1965). In the present study, ouabain has been shown to cause an increase in tissue sodium and a decrease in glucose accumulation. The effect on sodium levels seems to involve inhibition of the sodium extrusion process since sodium efflux is clearly inhibited. The partial reversal of the ouabain effect on the net influx of sodium cannot be readily explained and would merit further investigation. While ouabain may inhibit glucose accumulation through effects on sodium extrusion, with a consequent lowering of the external/internal sodium ratio, there remains a problem in explaining the inhibition of glucose uptake by ouabain in 2 min incubations. This inhibition of the initial rate of glucose absorption seems to be competitive in nature, and it seems probable that ouabain interacts directly with the transport system.

It might be suggested that ouabain might be hydrolyzed with the release of a competitively inhibitory sugar. However, we found the glycone moiety, rhamnose, to be without effect on glucose absorption. Thus, we may postulate that, in *Calliobothrium,* ouabain inhibits sugar transport directly by interacting with the membrane carrier and inhibits sugar accumulation indirectly by inhibiting the sodium extrusion mechanism. Further work is required to substantiate this interpretation.

Sodium in the external medium is required for glucose absorption by both larvae and adults of the cyclophyllidean tapeworm, *Taenia taeniaeformis.* In the absence of sodium, no glucose transport occurs and, at 12 and 46 m*M* sodium, glucose absorption is reduced about 70 and 25%, respectively (von Brand and Gibbs, 1966). If the response to sodium concentration is linear, as is the case with *Calliobothrium,* about 60 m*M* sodium would be required to maintain glucose transport at its highest level in *T. taeniaeformis.* This has not been examined experimentally, although von Brand and Gibbs (1966) found that in media containing 115 m*M* sodium, glucose absorption equaled that observed in media con-

taining 150 m*M* sodium. *Hymenolepis diminuta* also requires sodium for glucose transport. This will be discussed elsewhere (Fisher, in preparation).

The data on accumulation of various sugars by *Calliobothrium* verifies earlier reports (Read, 1957; Laurie, 1961) that this worm does not metabolize mannose or fructose. The worm is almost impermeable to these sugars. In the case of galactose, the worm seems to accumulate the sugar to higher levels than glucose can be accumulated. *Calliobothrium* has been reported to ferment galactose at rates almost equivalent to the rates of glucose fermentation (Read, 1957; Laurie, 1961). Thus, it may seem surprising that virtually no ^{14}C-galactose was incorporated into the polysaccharide of the worm. In a 60 min period, incorporation of ^{14}C-glucose into polysaccharide was 125-fold that of galactose. However, galactose is not glycogenic in the cyclophyllidean tapeworm *Hymenolepis diminuta*. Although starved *H. diminuta* showed a dramatic net glycogenesis when furnished with glucose, no significant net glycogenesis occurred when galactose was available (Read, 1967). Like *Calliobothrium, H. diminuta* transports galactose into the tissues by a specific mediated system and ferments this sugar, but galactose is not glycogenic.

A taxonomic note

Euzet (1954) reported that he had examined Linton's (1891) tapeworm material collected from dogfish at Woods Hole. Linton had identified these worms as *Calliobothrium eschrichtii* Beneden, but Euzet concluded that Linton's material represented a new species which he named *Calliobothrium lintoni*. However, the animal studied in the present investigation (as well as in previous studies by Read, 1957; Read *et al.*, 1960; Simmons, Read and Rothman, 1960; Laurie, 1961) is indeed *Calliobothrium verticillatum* (Rudolphi). We have found *C. lintoni* on several occasions in *Mustelus canis*. It is too small an organism for convenient physiological research.

Summary

1. Gas phase had no significant effect on glucose absorption or accumulation by *Calliobothrium*.

2. Optimum temperature for glucose accumulation is about 20° C.

3. Glucose transport was affected by pH, highest rates being observed at 8 to 9. This corresponds to the pH measured in that part of the spiral intestine inhabited by the strobilate worm.

4. Those sugars or glycosides which inhibited transport of glucose also stimulated efflux. Inhibitions produced by phlorizin, ouabain, galactose, maltose, and α-methylglucoside were found to be competitive in 2 min incubations.

5. The rate of glucose transport was a function of Na^+ in the external medium. Tissue Na^+ increased and K^+ decreased during glucose absorption.

6. Ouabain caused a net influx of Na^+ and this effect was attributed to the observed inhibition of Na^+ efflux.

7. Galactose was also accumulated by the worm but a negligible amount was incorporated in polysaccharide. Mannose, fructose, and 3–0-methylglucoside were not transported.

8. The data are discussed in terms of the specificity of the glucose transport mechanism. It is hypothesized that ouabain may inhibit two processes involved in glucose transport and accumulation.

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